RESPONSE OF SOIL MICROFAUNA TO TILLAGE METHODS AND CROPPING SYSTEMS IN HUMIC NITOSOLS OF EASTERN KENYA

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Abstract

In Eastern Kenya, appropriate soil tillage methods and cropping systems are among the strategies undergoing investigations for sustainable production. The crop productivity depends on availability of nutrients in the root zone. The enormous role of releasing and circulating of nutrients in the soil is enhanced by soil microfauna, which include bacteria, fungi and nematodes. It is essential to find out how microfauna respond to different tillage methods. The study was conducted at the Kenya Agricultural and Livestock Organization (KARLO-Embu) to determine the effects of tillage methods and cropping systems on bacteria, fungi and nematode populations. The treatments were tillage methods constituting furrows-ridges, zero tillage, conventional tillage; and cropping systems constituting maize beans intercrop, sole maize and sole beans. The treatments were laid out in a split-plot design. Results indicated that different tillage methods significantly ($p < 0.01$) influenced fungi and nematodes populations, while different cropping systems influenced nematodes populations. The influence of different tillage methods on bacteria population was non-significant ($F(2,97) = 2.02$, $p = 0.138$) as well as different cropping systems ($F(2,97) = 0.76$, $p = 0.469$). Interaction of 'maize bean cropping system' and 'furrow and ridges tillage method' resulted in highest fungi and nematodes population signifying that it could be a promising strategy for microfauna's conservation in the farms in humic Nitisols of Eastern Kenya.

Keywords: bacteria, fungi, furrows and ridges, nematodes, zero tillage

Introduction

Food insecurity in Kenya has led to many studies geared towards increase in agricultural production to satisfy increasing Kenyan population (FAO et al., 2012). In Eastern Kenya, appropriate soil tillage methods and cropping systems are among the strategies undergoing investigations for sustainable yields production (Micheni et al., 2011). The crop growth and yields production depend on availability of nutrients in the root zone (Sanginga and Woomer, 2009). The enormous role of releasing and circulating of nutrients in the soil is enhanced by soil microfauna which include bacteria, fungi and nematodes population (Njeru et al., 2012). Some bacteria colonize plants and proceed to promote plant growth and yield through direct production of phytohormones (Ngamau et al., 2014). The humic Nitisols are the most common type of soil in upper mid lands of Eastern Kenya and it has moderate to high inherent fertility (Gitari and Friesen, 2001; Jaetzold et al., 2006). Various studies have been successfully tried on soil tillage and cropping systems with fewer negative consequences on the environment (Kassam et al., 2014; Temesgen et al., 2009; Vanlauwe et al., 2014).

Appropriate tillage methods especially conservation tillage and cropping system such as intercropping are capable of building up sustainable soil system that would increase land productivity (Muraya et al., 2006; Watts-Padwick, 1983). Soil tillage methods such as zero tillage and furrows and ridges, and cropping systems such as intercropping are among the principles considered along with soil cover, in conservation agricultural systems (Kaumbutho and Kienzle J., 2007; Kerte´sz et al., 2008). Soil biology is a key component and should be considered when improving soil productivity (Clapperton, 2014). It is essential to find out how microfauna respond to different tillage methods (Six et al., 2004). This study was conducted in the Kenya Agricultural and Livestock Organization (KARLO-Embu) farm situated on the Eastern Kenya, to determine the effects of tillage methods and cropping systems on bacteria, fungi and nematode populations as a part of a major study that was investigating the effect of different farming systems on and maize and bean yields. The study spanned four seasons from 2011 short rains to 2013 long rains. The sampling for microfauna was done in the fourth season (2013 long rain).

Materials and Methods

Study Site

The study was conducted at the Kenya Agricultural and Livestock Research Organization (KARLO- Embu) farm on the Eastern slopes of Mt. Kenya at 00° 33.18'S; 037° 53.27'E; 1420 m above sea level and in the upper midlands (UM_3) zone. The region receives 1250 mm average annual bimodal rainfall and temperatures range from 21-28°C and 16 - 21°C mean maximum and minimum, respectively (Jaetzold et al., 2006). The two rainy seasons are the long rains (LR) lasting from March to August, and short rains (SR) from October to January (Jaetzold et al., 2006). About 65% of the rains fall during the LR and in some years end in July-August with scanty showers (Micheni et al., 2013). The soils are dominated by humic Nitisols (Jaetzold et al., 2006).

Test crops and planting densities

The test crops were maize (*var*. DK 8031) and common bean (Embean-14 or *Mwende*). DK 8031 is a medium maturity hybrid taking up to 135 days from emergence to physiological maturity in medium altitude zones (approximately 1350 m above sea level). Embean-14 is a determinate bush bean with potential grain yield of 2.5 t ha^{-1} $season^{-1}$ in upper midland zones. The variety takes approximately 95 days from emergence to physiological maturity. Maize and beans were sown at the on-set of the rains to effectively make use of the seasonal rainfall.

Experimental design

Treatments consisted of tillage methods (TM) and cropping systems (CS) (Table 1) laid in a split-plot design and replicated thrice. TM (furrows and ridges (FR), zero tillage (ZT) and conventional tillage (CVT) formed the main plots while CS (sole maize (SM), sole beans (SB) and maize beans intercrop (MB) made up the sub-plots. Two (2) meter buffer paths separated the blocks to guard the treatment effects from spilling over. One (1) meter paths on the other hand separated the sub-plots, and provided space for field operations and data collection.

Table 1. List of treatments in the main plots (tillage methods) and sub-plots (cropping systems).

	Treatments		Abbreviation
Main Plot Treatment	Tillage methods (TM)	Zero tillage	ZT
		Furrows-ridges	FR
		Conventional tillage	CVT
Sup-plot Treatment	Cropping system (CS)	Sole maize	SM
		Sole bean	SB
		Maize + bean intercrop	MB

TM = tillage method; CS = cropping system; RM; residue management; ZT = zero tillage; FR = furrows-ridges; CVT = convetional tillage; SM = sole maize; SB = sole bean; SM = sole maize; MB = maize and bean intercrop.

Main plots treatments

Conventional tillage (CVT): Plots were conventionally prepared using hand-hoes to till the land and achieve weed-free seedbed for maize and beans. Planting holes were made at the time of trial establishment. Two weed control events were conducted manually using hand tools (machete and hoes) within a given season. The first weeding was done 1 – 2 weeks after crop emergence and the second was conducted in approximately one and half months after the first weeding.

Zero tillage (ZT): This is a conservation agriculture method where no rigorous land tillage was done; instead, only seeding holes enough to hold seed and fertilizer material were made at the beginning of every season. Weeds were controlled using pre and post-emergence herbicides. Roundup *(Glyphosate)*, a post-emergence herbicide, was applied at the rate of 3.0 liters (L) ha^{-1} to kill weeds at the beginning of the seasons. Dual Gold (960 g L^{-1}*Metolachlor*), a pre-emergence herbicide, was applied at the rate of 2.0 L ha^{-1} on relatively moist soil surface after planting but before emergence of crops and weeds. One month after the crop emergence, selective *Basagran* post-emergence herbicide was applied at the rate of 2.0 Lha^{-1} to manage actively growing grass and broad-leafed weeds in maize-bean intercrop. The herbicide is effective mainly through contact action and therefore care was taken to have all weeds thoroughly covered with the herbicide sprays while avoiding maize and bean leaves.

Furrows-/ridges (FR): This was also a conservation agriculture method where furrows-ridges were made at a spacing of 75 cm apart during the time of the trial establishment. Like the case of ZT (above), weeds were managed using pre- and post-emergence herbicide(s) as was necessary.

Sub-plot treatments

Sole maize (SM): Maize spacing was 75 cm between rows and 50 cm within. Three seeds were sown per hill and thinned one week after crop emergence to two (2) plants per hill, to give a plant population of 53,333 plants ha^{-1}.

Sole bean (SB): Sole bean were spaced at 50 cm between rows and 15 cm within rows while maintaining one (1) plant per hill giving plant population of 133,330 plants ha^{-1}.

Maize bean intercrop (MB): Maize spacing was maintained like in maize sole crop but bean spacing was slightly adjusted to 50 cm between rows and 20 cm within rows and two (2) plants per hill. This gave plant population of 133,330 plants ha^{-1}, which was the same as in SB configuration. This was done to minimize confounding effects due to plant population differences.

Fertilizer application
Irrespective of tillage methods, SM, SB and MB received 60, 20 and 80 kg N ha^{-1} and 60, 51 and 111 kg P_2O_5 $ha^{-1}$from $[N_{23}P_{23}]$,$[N_{18}P_{46}]$ and combination of $[N_{23}P_{23}]$+$[N_{18}P_{46}]$ fertilizer materials respectively.

Data collection
Soil sampling
Collection of the soil samples for determination of microfauna was done in the fourth season of the experiment. Soil samples were taken at maize silking stage and just after bean harvesting from within the net plots of the sub-plots during 2013 long rains. Samples were taken between the rows to maximise the collection of free-living microfauna, which are mainly beneficial (Zhan and Sun, 2012). Three soil sub-samples were taken in a zigzag sampling method from every plot, at 0 - 20 cm soil depth using sterilized narrow bladed trowel. The sub-samples were then mixed per plot to make a composite sample in every plot. The soil samples were put in a well labelled plastic bag and mixed thoroughly but gently and placed in a cool box with ice to prevent them from heating up or drying out. The cool boxes were immediately transferred to the laboratory for extraction and enumeration of bacteria, fungi and nematode populations.

Laboratory microbial enumeration
Bacteria and fungi: Each soil sample was carefully mixed with a spatula in the sampling bottle. One gram of each soil sample was weighed on a sterile aluminium foil and immediately transferred to a test tube containing 9ml of sterile distilled water. The mixture was gently homogenized using a vortex shaker for 30 seconds after which the soil suspension was aseptically diluted serially by adding 1ml of the soil suspension to 9 ml test tube of sterile distilled water. Each time the preparation was shaken and 1ml of aliquots rapidly transferred to another 9 ml tube. Dilution ratios included: 10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. 100µl (0.1ml) aliquot from dilution 10^{-6} was aseptically transferred to plates containing nutrient agar (NA) and potato dextrose agar (PDA) for bacteria and fungi, respectively. The aliquot was then spread over the plate surface with a sterile glass rod in laboratory hood. The plates were inverted and incubated in the dark at 25°C for 72 hours, after which counting of colony forming units (cfu)/ml of bacteria and fungi was done.

Nematode: A 10 cm length rubber tubing was attached to the funnel stem and tubing clumped on. The funnel was mounted on ring stand, and then water was added to the funnel to two-thirds full and a wire-mesh basket placed on top to support tissue from falling off. A 50 cm^3 mixed and sieved soil subsample was spread evenly on tissue, the edges of the tissue were folded and the funnel was filled with water such that water level was about 5 mm above wire-mesh. Water and soil were not allowed to lose contact during extraction period to prevent dehydration. Hence, water was added as needs be. Temperature was maintained between 22 - 25° C which is usually conducive for nematode development (Barker, 1985). After 48 hours nematodes recovered were extracted by releasing 20 ml of water from stem of funnel into a counting dish. Counting of nematodes in their active stages was done with the help of light microscope.

Data Analysis
Due to the expected variability in microfauna (bacteria, fungi and nematodes) data, the normality test was first applied using Shapiro-Wilk test, Kolmogorov-Smirnov test, Anderson-Darling test and Cramér-von Mises using SAS 9.2 procedure. The last three tests were used because the population mean and standard deviation of the microfauna (bacteria, fungi and nematode) were not known and were to be estimated from the data. The data was then subjected to the analysis of variance (ANOVA), using SAS 9.2 for windows following General

Linear Model (GLM) procedure (p = 0.05). Maize and bean yield data was less variable and was also subjected to ANOVA. Where significant differences were detected among the treatment, the means were compared and separated using Least Significant Difference at p = 0.05 (Gomez and Gomez, 1984).

Results

All p-values obtained from normality tests for bacteria and fungi were above alpha (α) level of 0.05, signifying that the data came from a normally distributed population (Table 2). The p-values for nematodes were too low indicating that there was a lot of variation in their population. Indeed, Cramér-von Mises test, judged the nematode data as just equal to α (0.05). Nematode data was however analysed using ANOVA just as for bacteria and fungi, because other normality tests yielded p-values greater than alpha (0.05) (Table 2).

Tillage methods and cropping systems returned an F ratio of $F(2,97) = 4.99$, $p < 0.01$ and $F(2,97) = 10.46$, $p = <0.01$ respectively, indicating that at least one or more categories of microfauna studied (bacteria, fungi and nematodes) were influenced by either tillage methods or cropping systems or both.

Further investigation revealed that bacteria population ($M = 2.51 \times 10^8$, $SD = 4.3 \times 10^6$) did not vary neither within different tillage methods (TM) ($F(2,97) = 2.02$, $p = 0.138$) nor within different cropping systems (CS) ($F(2,97) = 0.76$, $p = 0.469$) (Table 3). Bacteria populations resulting from interactions of the three cropping systems (CS) (maize bean (MB) intercrop, sole bean (SB) and sole maize (SM)) and the three tillage methods (CVT, FR and ZT) were similarly non-significant (F ratio of $F(2,97) = 1.62$, $p = 0.175$), (Fig. 1).

Table 2. The List of p-values from normality test computed using SAS 9.2 procedure

Variate	Test	--Statistic---		--------P value------			
Bacteria	Shapiro-Wilk	W	0.993	Pr	<	W	0.84
	Kolmogorov-Smirnov	D	0.053	Pr	>	D	>0.15
	Cramer-von Mises	W-Sq	0.042	Pr	>	W-Sq	>0.25
	Anderson-Darling	A-Sq	0.230	Pr	>	A-Sq	>0.25
Fungi	Shapiro-Wilk	W	0.987	Pr	<	W	0.39
	Kolmogorov-Smirnov	D	0.056	Pr	>	D	>0.15
	Cramer-von Mises	W-Sq	0.049	Pr	>	W-Sq	>0.25
	Anderson-Darling	A-Sq	0.341	Pr	>	A-Sq	>0.25
Nematode	Shapiro-Wilk	W	0.980	Pr	<	W	0.11
	Kolmogorov-Smirnov	D	0.079	Pr	>	D	0.1
	Cramer-von Mises	W-Sq	0.126	Pr	>	W-Sq	0.05
	Anderson-Darling	A-Sq	0.729	Pr	>	A-Sq	0.06

P values higher than α = 0.05 indicates the population was normally distributed.

Table 3. Means for bacteria, fungi and nematode within classes of tillage methods, cropping system

Class	Treatment	N	Bacteria	Fungi	Nematode	Maize	Bean
			cfu x 10^6/1g of soil		Counts/50cm^3 of soil	Yield (t/ha)	
Tillage method	Conventional tillage	36	261.44^a	23.50^c	139.47^b	4.22^b	2.22^a
	Zero tillage	36	248.28^a	33.25^b	90.43^c	3.88^c	1.92^a
	Furrows-ridges	36	242.03^a	50.44^a	150.89^{ab}	4.64^a	2.29^a
Cropping system	Maize-bean intercrop	36	254.44^a	39.361^b	170.56^a	4.23^b	2.11^a
	Sole maize	36	253.75^a	36.89^b	128.42^{bc}	4.27^b	-
	Sole bean	36	243.56^a	30.944^b	79.31^c	-	2.18^a

Means with the same letter within the same column are not significantly different (lsd p = 0.05)**.** cfu = colony forming unit. N= Observations.

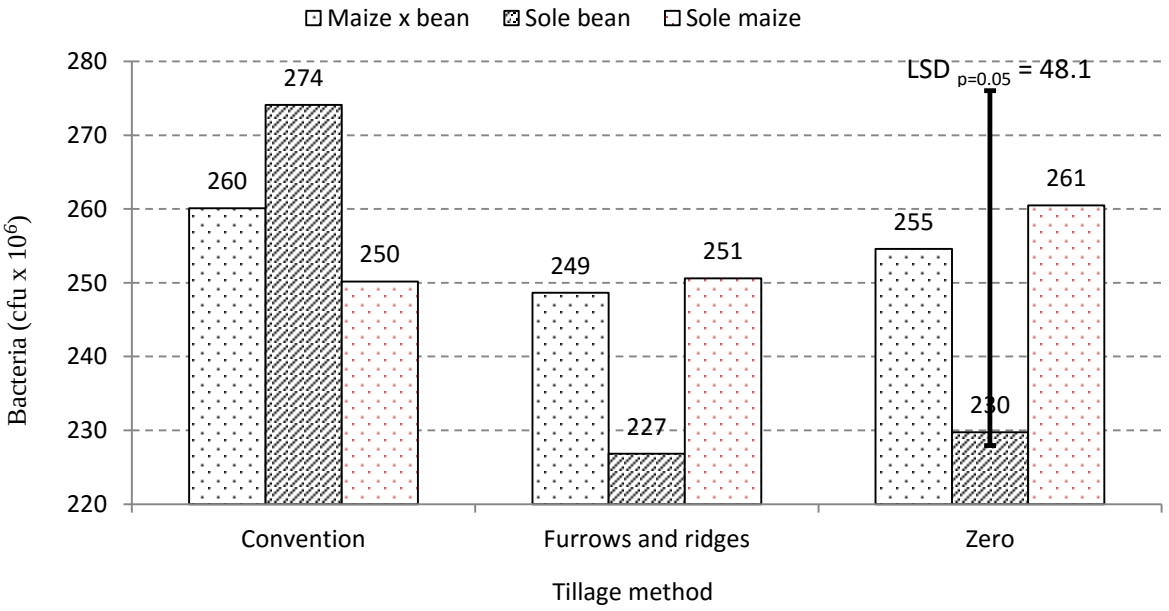

Fig. 1. Bacteria population (cfu x 10^6/1g of soil) based on tillage method and cropping systems. Neither tillage method nor cropping system had significantly different populations of bacteria. LSD – least significant difference.

Fungi population was significantly influenced by tillage methods $F(2,97) = 7.63$, $p < .01$. Conventional tillage (CVT) (M = 2.35 × 10^7, SD = 8.1 × 10^6) had the lowest population while furrow and ridges (FR) had highest (M = 5.04 × 10^7, SD = 5.73 × 10^6). Different cropping

systems did not influence the fungi population $F(2,97) = 0.77$, $p = 0.467$ (Table 3). The interaction of TM and CS generated an F ratio of $F(2,97) = 4.17$, $p < 0.01$, indicating that at least one of the combination among TM (CVT, FR, ZT) and CS (SM, SB and MB), significantly influenced fungi populations (Table 4). The most remarkable difference in fungi population was between sole bean under conventional tillage and sole bean under furrows and ridges, the later yielded significantly (p=0.05) higher fungi population (Fig. 2).

The effect of tillage on nematodes population yielded an F ratio of $F(2,97) = 4.30$, $p = 0.016$, indicating that the populations of nematodes in CVT, FR and zero tillage (ZT) methods differed significantly. ZT had the lowest nematodes populations (M = 87.9, SD =72) while FR had highest (M = 150.9, SD = 144). The effect of the cropping systems yielded an F ratio of $F(2,97) = 7.97$, $p < .01$, signifying that the populations of nematodes in different CS (SM, SB and MB) were significantly different. SB had lowest nematodes populations (M = 79.3, SD = 57) while MB had the highest population (M = 170.6, SD = 137) (Table 3). The interaction of TM and CS generated an F ratio of $F(2,97) = 5.62$, $p < 0.01$ on nematodes population, indicating that at least one of the combination among TM (CVT, FR, ZT) and CS (MB, SB and SM) significantly influenced nematodes populations (Table 4). The nematode populations in SB did not differ across the three tillage methods. However, there were differences in populations of nematodes present in SM and MB under all three tillage methods. Under CVT nematodes populations was highest in SM, under FR and ZT the population was highest in MB (Fig.3).

Table 4. Means for bacteria, fungi and nematode populations under tillage methods and cropping system interactions

Class	**N**	**Bacteria**	**Fungus**	**Nematode**	**Maize**	**Bean**
Tillage × Cropping System		cfu x 10^6/g of soil		Counts/50cm^3 of soil	Yield (t/ha)	
CVT × Sole bean	12	274.08^{a}	16.92^{c}	87.50^{bc}	-	2.27^{a}
ZT × Sole maize	12	260.50^{a}	35.17^{ab}	69.17^{c}	3.94^{c}	-
CVT × Maize bean	12	260.08^{a}	28.58^{ab}	151.67^{abc}	4.19^{b}	2.17^{a}
ZT × Maize bean	12	254.58^{a}	38.75^{ab}	131.25^{bc}	3.81^{c}	1.85^{a}
FR × Sole maize	12	250.58^{a}	50.50^{a}	136.83^{abc}	4.61^{a}	-
CVT × Sole maize	12	250.17^{a}	25.00^{ab}	179.25^{ab}	4.24^{b}	-
FR × Maize bean	12	248.67^{a}	50.75^{a}	228.75^{a}	4.68^{b}	2.31^{a}
ZT × Sole bean	12	229.75^{a}	25.83^{ab}	69.09^{c}	-	1.98^{a}
FR × Sole bean	12	226.83^{a}	50.08^{a}	87.08^{bc}	-	2.28^{a}

Means with the same letter within the same column are not significantly different (lsd $p = 0.05$)**.** cfu = colony forming unit, N= Observations, FR = Furrows and ridges, CVT = Convention tillage, ZT = Zero tillage

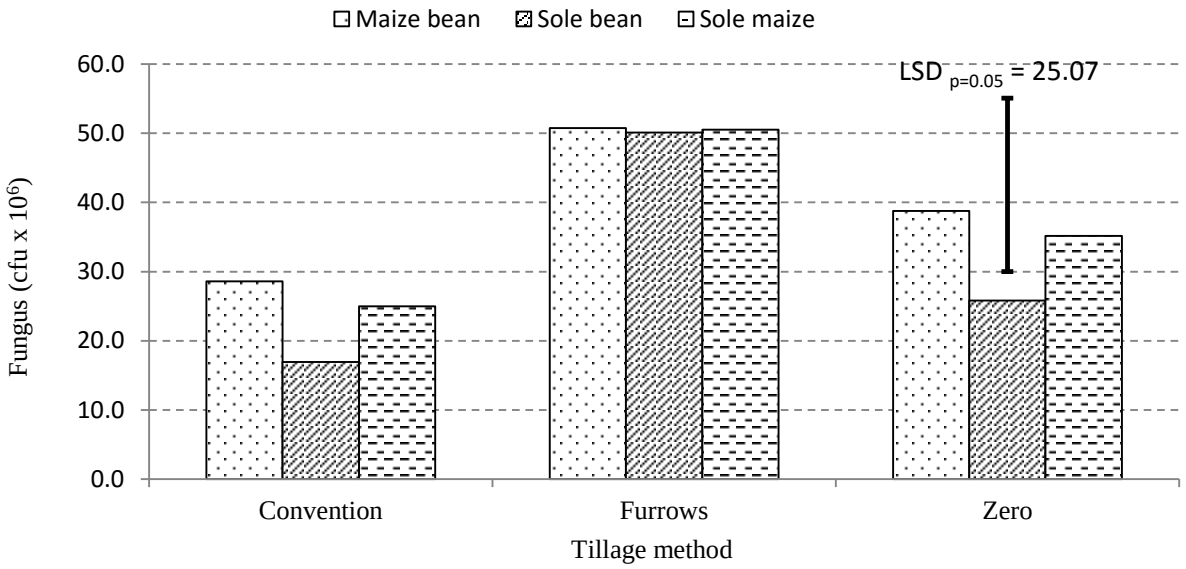

Fig. 2. Fungi population (cfu x 10^6/1g of soil) based on interactions between tillage method and cropping systems. Sole bean under conventional tillage yielded fungi population that was significantly (p=0.05) lower than that yielded under furrows and ridges.

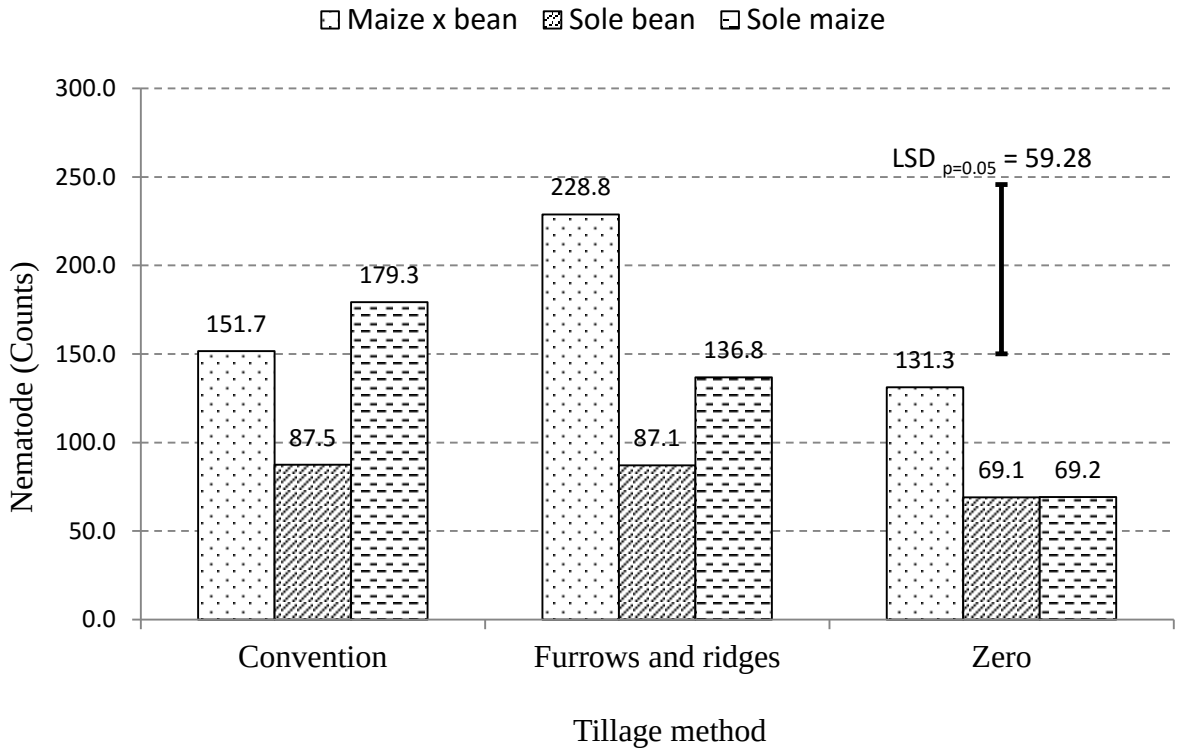

Fig. 3. Nematode population (counts/50cm^3 of soil) based on tillage method and cropping system. Nematode population shows a lot of dynamisms within tillage methods and cropping systems.

Maize yield under different TM generated an *F* ratio of $F(2,97) = 9.54$, $p < 0.01$, implying that tillage methods significantly influenced maize yield. On the other hand CS yielded an an *F* ratio of $F(2,97) = 0.06$, $p = 0.81$, signifying that cropping system did not influence maize yield

(Table 3). On bean yield, the influence of TM ($F(2,97) = 3.63$, $p = 0.051$), CS ($F(2,97) = 0.21$, $p < 0.65$), and their interactions were non-significant (Table 3). The interaction of the TM and CS on maize yield returned an F ratio of $F(2,97) = 20.08$, $p < 0.01$, indicating that there was significant differences in the yield due to interactions of TM and CS (Table 4).

Discussion

The fact that sampling was carried out between rows as compared to the roots contact zone, means that free living microfauna were targeted. Most of these have been found to be beneficial to plants (Campos-Herrera et al., 2012; Gebremikael et al., 2014; Sinha et al., 2016; Zhan and Sun, 2012). The soil microfauna' categories observed in current study included bacteria, fungi and nematodes. Similar categories have been found to dominate the soil microfauna in other studies (Treonis et al., 2010; Zhang et al., 2015).

Bacteria

As expected, bacteria population was highest in the soil compared to fungi and nematodes in cultivated landscapes, a trend that has been observed in previous study where bacteria was found to form larger percentage of microfauna in the soil (Njeru et al., 2012). Large population of bacteria in the soil may have been responsible for their stability regardless of disturbance due to tillage methods and cropping systems (Ekschmitt and Griffiths, 1998). Bacteria play important role in soil nitrification and hence their higher population was good for the general soil health (Sherwood and Uphoff, 2000). Non-significance differences in bacteria population in all treatments indicated that bacteria within the same geographical site are likely to be less dynamic (Rosa et al., 2014). Bacteria could vary if comparison was between soils covered by vegetation and the ones not covered (Qian et al., 2015), but the current study focused on cultivated land all of which had crop established in all plots, hence soil was mulched by live vegetation.

Fungi

FR resulted in relatively higher fungi populations compared to other tillage methods probably due to higher moisture levels in the furrows (Zhao et al., 2015). Fungi are known to be higher under moist conditions (Njeru et al., 2012). The remarkable observation was that the maize yield was significantly highest where fungi were significantly highest, pointing to possible symbiosis (Singh, 2015) and indicating that the fungi found here were not harmful to crop. The population of fungi was second in highest from bacteria. This confirms previous studies where fungi were found to form the second largest portion in percentage in the soil microfauna after bacteria (Yuan et al., 2015). Conventional tillage had lowest fungi population compared to zero tillage and furrows and ridges, this was associated with expected higher soil carbon. Earlier study have revealed that zero tilled soil contain 9% higher carbon than tilled soil and have found this to attract more microbial community (Mangalassery et al., 2015).

Nematodes were the most variable among the microfauna. This indicated that nematodes were very dynamic across the tillage and cropping systems. This confirms earlier findings that nematodes are vulnerable to disturbance (Park et al., 2013). It was noted that cropping system influenced nematodes population probably indicating that some crops or crop combination may result in exudates favourable to nematodes (Larson et al., 2000). In the current study, sole beans limited nematodes population. The reason for this limitation is not well known, nonetheless, earlier study also found that nematode abundance was lower in soybean system (Ito et al., 2015). These findings may be signifying that some legumes growing solely may not be favourable to nematodes. An intercrop of maize and bean (MB) however,

resulted in highest nematode population, higher than one found in sole maize system. Correspondingly, maize-bean intercrop system gave the highest maize and bean yield, indicating that large number of nematodes found were also beneficial, a similar trend was observed when legume (Mucuna) was intercropped with maize (Blanchart et al., 2006). The reason for this surge in nematode populations remains unknown, but advances speculation that maize bean interaction may have favoured the free-living nematodes. This raises hope that intercrop could offer a solution to a challenge posed in earlier study on how to develop sustainable management systems that could be a vanguard of the soil health (Doran and Zeiss, 2000). FR had the highest nematode population among the tillage methods. Notably, this is the tillage where highest moisture levels were expected, given that many studies have revealed that furrows result in higher moisture retention (Baudron et al., 2012; Karunakaran and Behera, 2016). The findings were therefore in tandem with previous studies, which have shown that microfauna communities in soil increases with increase in soil moisture content (Yuan et al., 2015). Zero tillage produced lowest nematode population and this was associated with expected higher soil bulk density. The higher the bulk density the lower the nematode population (Ito et al., 2015).

Conclusions and Recommendations

Current study revealed that different components of soil microfauna respond differently to tillage methods and cropping systems in humic Nitisols of Eastern Kenya. Bacteria population was not significantly influenced by tillage methods and cropping system. Besides giving the highest maize and bean yields, the interaction of 'furrows and ridges tillage system' and 'maize bean intercrop cropping system' also resulted in highest fungi and nematodes population, signifying that it could be a promising strategy for microfauna's conservation in the farms in humic Nitisols of Eastern Kenya.

Acknowledgements

The authors wish to thank the International Maize and Wheat Improvement Center (CIMMYT) and the Kenya Agricultural and Livestock Research Organization (KALRO) for financial and technical support in this research. Also acknowledged is the horticulture department of Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya for collaboration and laboratory support.

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